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Inactivation of polyphenol oxidase from Pacific white shrimp by dense phase carbon dioxide

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ABSTRACT

The inactivation of polyphenol oxidase (PPO) from Pacific white shrimp exposed to dense phase carbon dioxide (DPCD) treatment was investigated. PPO activity showed a dramatic loss at 4–25 MPa and 37 °C. At the lower pressure (4–15 MPa), the experimental data of inactivation followed the first-order reaction kinetic model, the pressure sensitivity (Z_P) of the kinetic parameters was 49.02 MPa and the activation volume ($\triangle V^{\neq}$) was −120.88 cm^3/mol. At the higher pressure (20 and 25 MPa), the experimental data of inactivation followed the biexponential kinetic model. The kinetic rate constant k_F and k_S of fast and stable fractions were 2.45 and 0.08 min^{-1}, respectively. The decimal reduction time D_F and D_S were 0.94 and 29.43 min at 25 MPa and 37 °C, respectively. After DPCD treatment, the loss activity of PPO had no restoration storing for 6 days at 4 °C. The results of SDS-PAGE and activity staining also showed that DPCD treatment had the obvious inhibitory effect on PPO from Pacific white shrimp. The PPO activity in vivo was easier to be inactivated than that in crude PPO extracts under the same DPCD treatment conditions.

Industrial relevance: There is a growing interest in non-thermal pasteurization methods, which could retain food's freshlike physical, nutritional, and sensory qualities. Pacific white shrimp accounts for 90% of the global aquaculture shrimp production, they are becoming increasingly popular. However, enzymatic browning of shrimp has been of great concern to food scientists and food processors. Dense phase carbon dioxide (DPCD) may be an adequate tool to obtain high quality since PPO activity could not be inactivated totally by high pressure under 400 MPa yet. The present work deals with the inactivation of PPO from Pacific white shrimp exposed to DPCD treatment in order to explore the feasibility of shrimp by DPCD process.

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1. Introduction

Food processing aims for preservation of foods with acceptable quality, security assurance and extending shelf life by minimizing the changes in natural color, taste, flavor and texture (Spilimbergo & Bertucco, 2003). Thermal pasteurization is a well known technique for food processing, however, drawbacks such as destruction of heat-sensitive nutrients, changes in physical, mechanical, and optical properties of the material involved in the treatment are difficult to be avoided. In addition, the technique is hard to balance between overheating (reducing the food's organoleptic properties) and under-heating (leading to unsafe and low-quality food products) (Damar & Balaban, 2006; Garcia-Gonzalez et al., 2007; Zhang, Davis, Matthews, Drews, LaBerge and An, 2006). Due to the increased consumer demand for nutritious and fresh-like food products with security assurance, non-thermal processing has been under intense investigation by many researchers as an alternative or complementary process to traditional thermal methods (Gui et al., 2007; Spilimbergo & Bertucco, 2003).

Dense phase carbon dioxide (DPCD) is a non-thermal pasteurization method that affects microorganisms and enzymes through molecular effects of CO_2 under pressures below 50 MPa. The temperature of DPCD treatment is low enough to avoid the thermal effects of traditional pasteurization to food products and retain their freshlike physical, nutritional, and sensory qualities (Damar & Balaban, 2006). Some research had shown that DPCD treatment had significant lethal effect on microorganisms in liquid and solid foods (Garcia-Gonzalez, Geeraerd, Elst, Van Ginneken, Van Impe and Devlieghere, 2009; Meujo, Kevin, Peng, Bowling, Liu and Hamann, 2010; Spilimbergo, Elvassore, & Bertucco, 2002; Zhang et al., 2006). Meanwhile, some research had also showed that DPCD treatment could inactivate pectinesterase, lipoxygenase, polyphenol oxidase (PPO) and peroxidase (POD) either in pure enzymatic solutions or in real food systems such as fruit juice (Balaban, Arreola, Marshall, Peplow, Wei and Cornell, 1991; Chen, Balaban, Wei, Marshall, & Hsu, 1992; Gui et al., 2007; Liu, Gao, Peng, Yang, Xu and Zhao, 2008; Liu, Gao, Xu, Hao, Liu and Wang, 2010; Tedjo, Eshtiaghi, & Knorr, 2000).

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Balaban et al. (1991) reported that pectinesterase (PE) activity in orange juice could be inactivated with supercritical CO_2 below temperatures necessary for thermal inactivation. Inactivation kinetics showed *D* values was 10 min at 31 MPa and 60 °C, while 2673 min at atmospheric pressure and 40 °C. However, many reactions catalyzed by enzyme had been performed in supercritical CO_2 (Habulin & Knez, 2001; Wimmer & Zarevúcka, 2010). The stability and the activity of enzymes exposed to DPCD treatment are affected by the type and source of the enzyme, DPCD treatment conditions such as pressure, temperature, time, and properties of the suspending medium (Chen et al., 1992; Liu et al., 2008; Zhou, Zhang, Hu, Liao, & He, 2009).

Enzyme inactivation by DPCD could be due to the three-dimensional structure changes of enzymes and inhibitory effect of molecular CO_2 on enzyme activity (Damar & Balaban, 2006; Wimmer & Zarevúcka, 2010). Ishikawa, Shimoda, Yonekura, and Osajima (1996) studied the conformational changes of lipase, alkaline protease, acid protease and glucoamylase by DPCD, the residual α-helix contents of which were 62.9, 31.3, 37.6, and 12.4%, respectively. Chen et al. (1992) reported that the secondary structures of PPO from Florida spiny lobster, brown shrimp and potato were changed by high-pressure CO_2 treatment.

Pacific white shrimp (*Litopenaeus vannamei*) accounts for 90% of the global aquaculture shrimp production. Unfavorable color change associated with melanosis on the surface of shrimp products has been of great concern to food scientists and food processors. Melanosis is triggered by a biochemical mechanism which oxidizes phenols to quinones by polyphenol oxidase, followed by non-enzymatic polymerization of the quinones, giving rise to dark pigments of high molecular weight (Nirmal & Benjakul, 2009). Although the pigmentation seems to be harmless to consumers, it drastically reduces the product's market value, leading to considerable financial loss. Several methods, such as the addition of chemicals and high pressure treatment, have been extensively studied for their effectiveness in inhibiting PPO activity (Gomes & Ledward, 1996; Montero, Ávalos, & Pérez-Mateos, 2001; Nirmal & Benjakul, 2010). However, the toxicity of chemicals such as sulphites and their derivatives has been getting more and more attention by consumers (Chen et al., 1992; Nirmal & Benjakul, 2009). PPO activity could not be inactivated totally under 400 MPa yet. It was reported that high pressures (400 MPa, 10 min, 7 °C) activated the melanosis process on prawns (Gomes & Ledward, 1996; Montero, Ávalos,et al., 2001; Montero, Lopez-Caballero, et al., 2001).

In the paper, the inactivation of PPO from Pacific white shrimp exposed to DPCD treatment was investigated in order to explore the feasibility of shrimp by DPCD process.

2. Materials and methods

2.1. Materials

Pacific white shrimps (*L. vannamei*) with the average sizes of 55–60 shrimps/kg were purchased from the Dongfeng seafood wholesale market in Zhanjiang, China. The shrimps were kept in ice with a shrimp/ice ratio of 1:3 (w/w) and transported to the laboratory within 1 h. Upon arrival, shrimps were washed in cold water and stored in ice until used (not more than 5 h).

3-(3,4-Dihydroxyphenyl)-L-alanine(L-DOPA), Brij-35 were purchased from Sigma-Aldrich (St. Louis, MO, USA). High Molecular Weight-SDS calibration kits were purchased from Dingguo Co. (Beijing, China). All other chemicals were of analytical grade. The purity of CO_2 was 99.9%, which was purchased from Zhanjiang Oxygen Corporation (Zhanjiang, Guangdong Province, China).

2.2. Dense phase carbon dioxide (DPCD) treatment system

The continuous DPCD treatment apparatus was manufactured by Nantong Huaan Supercritical Extraction Co. Ltd., (Nantong, China). A schematic diagram of the DPCD treatment system was shown in Fig. 1 (Gui et al., 2007). The system consisted of a 1L stainless steel pressure vessel, temperature controllers, pressure gages, and two plunger-type pumps. The system pressure was controlled by a back-pressure regulator and indicated by pointer manometers. An electrical heating jacket was placed around the vessel. Another thermocouple, connected to a temperature controller, was to control and maintain a constant temperature. The pressure and temperature were controlled to an accuracy of ±0.4 MPa and ±0.5 °C, respectively.

2.3. Enzyme extraction

The cephalothoraxes (head with the viscera removed) of thirty shrimps were separated, pooled and powdered by grinding with liquid nitrogen in a Waring blender. The powder obtained was kept in polyethylene bag and stored at −80 °C. The isolation of PPO was carried out according to the method of Simpson, Marshall, and Otwell (1987) with a slight modification. The powder (150 g) was mixed with 450 mL 0.05 M sodium phosphate buffer, pH 7.2, containing 0.5 M NaCl and 0.2% Brij 35. The mixture was stirred continuously for 30 min at 4 °C, followed by centrifugation at 8000 *g* at 4 °C for 30 min using a refrigerated centrifuge (Hitachi, Kyoto, Japan). The supernatant was collected and precipitated with ammonium sulfate to obtain 40% saturation and allowed to stand for 30 min at 4 °C. After subsequent centrifugation at 12,500 *g* for 30 min at 4 °C, the pellet obtained was dissolved in a minimum volume of 0.05 M sodium phosphate buffer, pH 7.2 and dialysed against three changes of 4 L of distilled water. The insoluble materials were removed by centrifugation at 3000 *g* for 30 min at 4 and the supernatant was used as "crude PPO extracts".

2.4. Measurement of PPO activity

PPO activity was assayed using L-DOPA as a substrate according to the method of Simpson et al. (1987) with a slight modification. Measurements were made in a mixture of 0.3 mL of crude PPO extracts, 1 mL of 15 mML-DOPA in deionised water, 1 mL of 0.05 M phosphate buffer pH 6.0 and 1 mL of deionised water. The PPO activity was determined for 3 min at 25 °C by monitoring the formation of dopachrome at 475 nm using a UV-2550 spectrophotometer (Shimadzu, Kyoto, Japan). One unit of PPO activity was defined as an increase in the absorbance by 0.001 at 475 nm/min/mL. The blank was prepared by excluding the enzyme from the reaction mixture and deionised water was used instead.

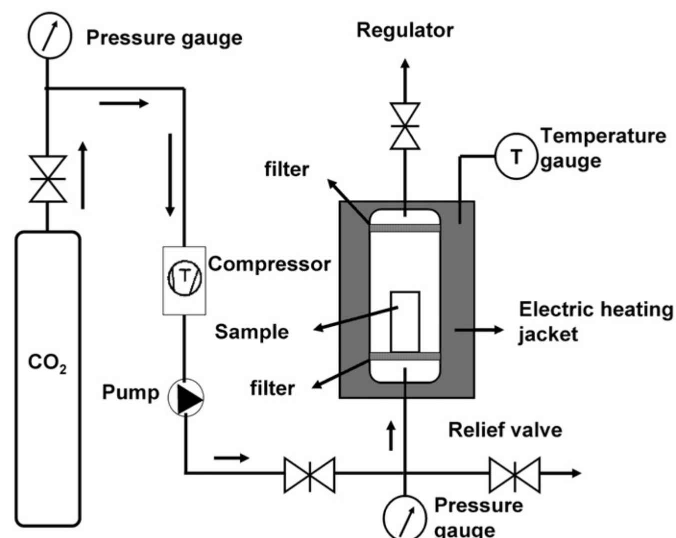

Fig. 1. Schematic diagram of DPCD processing equipment.

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